

WHAT IS CLAIMED IS:

1. A method for sterilizing a preparation of one or more digestive enzymes that is sensitive to radiation, said method comprising irradiating said preparation of one or more digestive enzymes with radiation for a time effective to sterilize said preparation of one or more digestive enzymes at a rate effective to sterilize said preparation of one or more digestive enzymes and to protect said preparation of one or more digestive enzymes from said radiation.
2. A method for sterilizing a preparation of one or more digestive enzymes that is sensitive to radiation, said method comprising:
  - (i) adding to said preparation of one or more digestive enzymes at least one stabilizer in an amount effective to protect said preparation of one or more digestive enzymes from said radiation; and
  - (ii) irradiating said preparation of one or more digestive enzymes with a suitable radiation at an effective rate for a time effective to sterilize said preparation of one or more digestive enzymes.
3. A method for sterilizing a preparation of one or more digestive enzymes that is sensitive to radiation, said method comprising:
  - (i) reducing the residual solvent content of said preparation of one or more digestive enzymes to a level effective to protect said preparation of one or more digestive enzymes from said radiation; and
  - (ii) irradiating said preparation of one or more digestive enzymes with a suitable radiation at an effective rate for a time effective to sterilize said preparation of one or more digestive enzymes.
4. A method for sterilizing a preparation of one or more digestive enzymes that is sensitive to radiation, said method comprising:

(i) reducing the temperature of said preparation of one or more digestive enzymes to a level effective to protect said preparation of one or more digestive enzymes from said radiation; and

(ii) irradiating said preparation of one or more digestive enzymes with a suitable radiation at an effective rate for a time effective to sterilize said preparation of one or more digestive enzymes.

5. A method for sterilizing a preparation of one or more digestive enzymes that is sensitive to radiation, said method comprising:

(i) applying to said preparation of one or more digestive enzymes at least one stabilizing process selected from the group consisting of

(a) reducing the residual solvent content of said preparation of one or more digestive enzymes,

(b) reducing the temperature of said preparation of one or more digestive enzymes, and

(c) adding at least one stabilizer to said preparation of one or more digestive enzymes; and

(ii) irradiating said preparation of one or more digestive enzymes with a suitable radiation at an effective rate for a time effective to sterilize said preparation of one or more digestive enzymes, wherein said at least one stabilizing process and the rate of irradiation are together effective to protect said preparation of one or more digestive enzymes from said radiation.

6. A method for sterilizing a preparation of one or more digestive enzymes that is sensitive to radiation, said method comprising:

(i) applying to said preparation of one or more digestive enzymes at least two stabilizing processes selected from the group consisting of

5 (a) reducing the residual solvent content of said preparation of one or more digestive enzymes,

10 (b) reducing the temperature of said preparation of one or more digestive enzymes, and

15 (c) adding at least one stabilizer to said preparation of one or more digestive enzymes; and

20 *Sub A1* (ii) irradiating said preparation of one or more digestive enzymes with a suitable radiation at an effective rate for a time effective to sterilize said preparation of one or more digestive enzymes, wherein said at least two stabilizing processes are together effective to protect said preparation of one or more digestive enzymes from said radiation and further wherein said at least two stabilizing processes may be performed in any order.

25 7. The method according to claim 3, 5 or 6, wherein said solvent is water.

30 8. The method according to claim 7, wherein said residual water content is reduced by the addition of an organic solvent.

35 9. The method according to claim 3, 5 or 6, wherein said solvent is an organic solvent.

40 *Sub A2* 10. The method according to claim 3, 5 or 6, wherein said preparation of one or more digestive enzymes is suspended in an organic solvent following reduction of said residual solvent content.

45 11. The method according to claim 1, 2, 3, 4, 5 or 6, wherein said effective rate is not more than about 3.0 kGy/hour.

50 12. The method according to claim 1, 2, 3, 4, 5 or 6, wherein said effective rate is not more than about 2.0 kGy/hr.

55 13. The method according to claim 1, 2, 3, 4, 5 or 6, wherein said effective rate is not more than about 1.0 kGy/hr.

14. The method according to claim 1, 2, 3, 4, 5 or 6, wherein said effective rate is not more than about 0.3 kGy/hr.

15. The method according to claim 2, 3, 4, 5 or 6, wherein said effective rate is more than about 3.0 kGy/hour.

5 16. The method according to claim 2, 3, 4, 5 or 6, wherein said effective rate is at least about 6.0 kGy/hour.

17. The method according to claim 2, 3, 4, 5 or 6, wherein said effective rate is at least about 18.0 kGy/hour.

18. The method according to claim 2, 3, 4, 5 or 6, wherein said effective rate is at least about 30.0 kGy/hour.

19. The method according to claim 2, 3, 4, 5 or 6, wherein said effective rate is at least about 45 kGy/hour.

20. The method according to claim 1, 2, 3, 4, 5 or 6, wherein said preparation of one or more digestive enzymes is maintained in a low oxygen atmosphere.

15 21. The method according to claim 1, 2, 3, 4, 5 or 6, wherein said preparation of one or more digestive enzymes is maintained in an atmosphere comprising at least one noble gas.

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22. The method according to claim 21, wherein said noble gas is argon.

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23. The method according to claim 1, 2, 3, 4, 5 or 6, wherein said preparation of one or more digestive enzymes is maintained in a vacuum.

20 24. The method according to claim 3, 5 or 6, wherein said residual solvent content is reduced by a method selected from the group consisting of lyophilization, drying, concentration, addition of solute, evaporation, chemical extraction, spray-drying and vitrification.

25. The method according to claim 3, 5 or 6, wherein said residual solvent content is less than about 15%.

26. The method according to claim 3, 5 or 6, wherein said residual solvent content is less than about 10%.

5 27. The method according to claim 3, 5 or 6, wherein said residual solvent content is less than about 3%.

28. The method according to claim 3, 5 or 6, wherein said residual solvent content is less than about 2%.

10 29. The method according to claim 3, 5 or 6, wherein said residual solvent content is less than about 1%.

15 30. The method according to claim 3, 5 or 6, wherein said residual solvent content is less than about 0.5%.

20 31. The method according to claim 3, 5 or 6, wherein said residual solvent content is less than about 0.08%.

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Cent* 32. The method according to claim 1, 2, 3, 4, 5 or 6, wherein at least one sensitizer is added to said preparation of one or more digestive enzymes prior to said step of irradiating said preparation of one or more digestive enzymes.

25 33. The method according to claim 1, 2, 3, 4, 5 or 6, wherein said preparation of one or more digestive enzymes contains at least one biological contaminant or pathogen selected from the group consisting of viruses, bacteria, yeasts, molds, fungi, prions or similar agents responsible, alone or in combination, for TSEs and single or multicellular parasites.

20 34. The method according to claim 2, 5 or 6, wherein said at least one stabilizer is an antioxidant.

35. The method according to claim 2, 5 or 6, wherein said at least one stabilizer is a free radical scavenger.

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36. The method according to claim 2, 5 or 6, wherein said at least one stabilizer is a combination stabilizer.

5 37. The method according to claim 2, 5 or 6, wherein said at least one stabilizer is a ligand.

38. The method according to claim 37, wherein said ligand is heparin.

39. The method according to claim 2, 5 or 6, wherein said at least one stabilizer reduces damage due to reactive oxygen species.

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40. The method according to claim 2, 5 or 6, wherein said at least one stabilizer is selected from the group consisting of: ascorbic acid or a salt or ester thereof; glutathione; 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; uric acid or a salt or ester thereof; methionine; histidine; N-acetyl cysteine; lipoic acid; sodium formaldehyde sulfoxylate; gallic acid or a derivative thereof; propyl gallate and mixtures of two or more thereof.

41. The method according to claim 40, wherein said mixtures of two or more additional stabilizers are selected from the group consisting of: mixtures of ascorbic acid, or a salt or ester thereof, and uric acid, or a salt or ester thereof; mixtures of ascorbic acid, or a salt or ester thereof, and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; mixtures of ascorbic acid, or a salt or ester thereof, uric acid, or a salt or ester thereof, and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; and mixtures of uric acid, or a salt or ester thereof; lipoic acid; sodium formaldehyde sulfoxylate; gallic acid or a derivative thereof; propyl gallate and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid.

*Sub A 5*  
42. The method according to claim 2, 5 or 6, wherein said at least one stabilizer is a dipeptide stabilizer.

43. The method according to claim 42, wherein said dipeptide stabilizer is selected from the 25 group consisting of glycyl-glycine (Gly-Gly), carnosine and anserine.

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44. The method according to claim 1, 2, 3, 4, 5 or 6, wherein said radiation is corpuscular radiation or electromagnetic radiation, or a mixture thereof.

45. The method according to claim 44, wherein said electromagnetic radiation is selected from the group consisting of radio waves, microwaves, visible and invisible light, ultraviolet light, x-ray radiation, gamma radiation and combinations thereof.

5 46. The method according to claim 1, 2, 3, 4, 5 or 6, wherein said radiation is gamma radiation.

47. The method according to claim 1, 2, 3, 4, 5 or 6, wherein said radiation is E-beam radiation.

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10 48. The method according to claim 1, 2, 3, 4, 5 or 6, wherein said radiation is visible light.

11 49. The method according to claim 1, 2, 3, 4, 5 or 6, wherein said radiation is ultraviolet light.

12 50. The method according to claim 1, 2, 3, 4, 5 or 6, wherein said radiation is x-ray radiation.

13 51. The method according to claim 1, 2, 3, 4, 5 or 6, wherein said radiation is polychromatic visible light.

14 52. The method according to claim 1, 2, 3, 4, 5 or 6, wherein said radiation is infrared.

15 53. The method according to claim 1, 2, 3, 4, 5 or 6, wherein said radiation is a combination of one or more wavelengths of visible and ultraviolet light.

54. The method according to claim 1, 2, 3, 5 or 6, wherein said irradiation is conducted at 20 ambient temperature.

55. The method according to claim 1, 2, 3, 4, 5 or 6, wherein said irradiation is conducted at a temperature below ambient temperature.

56. The method according to claim 1, 2, 3, 4, 5 or 6, wherein said irradiation is conducted below the freezing point of said preparation of one or more digestive enzymes.

57. The method according to claim 1, 2, 3, 4, 5 or 6, wherein said irradiation is conducted below the eutectic point of said preparation of one or more digestive enzymes.

5 58. The method according to claim 1, 2, 3, 5 or 6, wherein said irradiation is conducted at a temperature above ambient temperature.

59. A composition comprising at least one preparation of one or more digestive enzymes and at least one stabilizer in an amount effective to preserve said preparation of one or more digestive enzymes for its intended use following sterilization with radiation.

10 60. A composition comprising at least one preparation of one or more digestive enzymes, wherein the residual solvent content of said preparation of one or more digestive enzymes is at a level effective to preserve said preparation of one or more digestive enzymes for its intended use following sterilization with radiation.

15 61. The composition of claim 60, wherein said residual solvent content is less than about 15%.

62. The composition of claim 60, wherein said residual solvent content is less than about 10%.

63. The composition of claim 60, wherein said residual solvent content is less than about 5%.

64. The composition of claim 60, wherein said residual solvent content is less than about 2%.

20 65. The composition of claim 60, wherein said residual solvent content is less than about 1%.

66. The composition of claim 60, wherein said residual solvent content is less than about 0.5%.

67. The composition of claim 60, wherein said residual solvent content is less than about 0.08%.

68. The composition of claim 59 or 60, wherein said preparation of one or more digestive enzymes is glassy or vitrified.

5 69. The composition of claim 59 or 60, wherein said preparation of one or more digestive enzymes contains at least one enzyme selected from the group consisting of trypsin, glycosidases and sulfatases.

70. The composition of claim 60, wherein the total protein concentration of said preparation of one or more digestive enzymes is at least about 0.5%.

10 71. The composition of claim 60, wherein the total protein concentration of said preparation of one or more digestive enzymes is at least about 1%.

15 72. The composition of claim 60, wherein the total protein concentration of said preparation of one or more digestive enzymes is at least about 5%.

73. The composition of claim 60, wherein the total protein concentration of said preparation of one or more digestive enzymes is at least about 10%.

15 74. The composition of claim 60, wherein the total protein concentration of said preparation of one or more digestive enzymes is at least about 15%.

75. The composition of claim 60, wherein the total protein concentration of said preparation of one or more digestive enzymes is at least about 20%.

20 76. The composition of claim 60, wherein the total protein concentration of said preparation of one or more digestive enzymes is at least about 25%.

77. The composition of claim 60, wherein the total protein concentration of said preparation of one or more digestive enzymes is at least about 50%.

25 78. A method of treating a digestive enzyme deficiency in a mammal comprising administering to a mammal in need thereof an effective amount of a preparation of one or more

digestive enzymes which has been sterilized according to the method according to claim 1, 2, 3, 4, 5 or 6

71. *—* 4, 5 or 6. 71-<sup>1</sup> according to claim 78, wherein said mammal is a human.

79. The method according to claim 78, wherein said digestive enzyme deficiency is Fabry disease.

80. The method according to claim 78, wherein said digestive enzyme deficiency is Fabry disease.

81. The method according to claim 78, wherein said digestive enzyme deficiency is Hunter syndrome.

82. The method according to claim 78, wherein said digestive enzyme deficiency is Gaucher's disease. digestive

10 83. The method according to claim 78, wherein said preparation of one or more digestive  
— enzymes comprises  $\alpha$ -galactosidase.

84. The method according to claim 78, wherein said preparation of one or more digestive enzymes comprises iduronate-2-sulfatase.

85. The method according to claim 37, wherein said ligand is a substrate or substrate analog of at least one digestive enzyme contained in said preparation of one or more digestive enzymes.

Or at least one argument.

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